

**Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
Special Emphasis Panel**

Minutes of the Peer Review Panel Meeting on the Revised Up-and-Down Procedure: A

Test Method for Assessing the Acute Oral Toxicity Potential of Chemicals

Introduction

A public meeting of an independent peer review panel was convened on July 25, 2000, at the Sheraton Imperial-Crystal City in Arlington, VA, to review the Revised Up-and-Down Procedure (UDP). The purpose of this meeting was to evaluate the validation status of the UDP as a replacement for the conventional LD50 test (OECD TG401; EPA OPPTS 870.1100). The meeting was organized by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the NTP. A comprehensive report of the peer review panel is provided as an attachment to these minutes.

The following expert scientists served on the peer review panel:

- Curtis D. Klaassen, Ph.D., D.A.B.T., D.A.T.S., Head, Section on Toxicology, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS (Panel Co-Chair)
- Diane K. Gerken, D.V.M., Ph.D., D.A.B.T., D.A.B.V.T., Manager, Toxicology, Battelle, Columbus, OH (Panel Co-Chair)
- George Alexeeff, Ph.D., D.A.B.T., Deputy Director for Scientific Affairs, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.
- Bas J. Blaauboer, Ph.D., Associate Professor of Toxicology, Research Institute of Toxicology, Utrecht University, Utrecht, The Netherlands
- Kimberly Bonnette, M.S., LATG, Manager of Acute Toxicology, Springborn Laboratories, Inc., Spencerville, OH.
- Phil P.A. Botham, Ph.D., MRCPath, Section Head-Toxicity, Central Toxicology Laboratory, Zeneca, Ltd., Cheshire, United Kingdom
- Robert Condon, Ph.D., Consulting Biostatistician (Retired from the FDA Center for Veterinary Medicine), Myersville, MD
- Robert Copeland, Ph.D., Associate Professor, College of Medicine, Howard University, Washington, DC
- Wyman Dorough, Ph.D., Professor and Toxicologist, Mississippi State University, Starkville, MS

- Nancy Flournoy, Ph.D., Professor, Department of Mathematics and Statistics, American University, Washington, DC
- Charles Hastings, Ph.D., Manager of Toxicology, BASF Corporation, RTP, NC
- Wallace Hayes, Ph.D., D.A.B.T., D.A.T.S., Vice President for Corporate Product Integrity, The Gillette Company, Boston, MA
- Janice Kuhn, Ph.D., D.A.B.T., Group Leader, Toxicology, Stillmeadow, Inc., Sugar Land, TX
- John Reeve, M.S., National Manager (Toxicology and Residues), New Zealand Ministry of Agriculture and Forestry, Food Assurance Authority, ACVM Group, Wellington, New Zealand
- Robert Scala, Ph.D., D.A.B.T., D.A.T.S., Toxicology Consultant, Tucson, AZ
- Nigel Stallard, Ph.D., Senior Research Fellow, Medical and Pharmaceutical Statistics Research Unit, The University of Reading, Early Gate Reading, United Kingdom
- Arthur A.J. van Iersel, Ph.D., Senior Toxicologist, National Institute of Public Health and the Environment, Centre for Alternatives to Animal Testing, Bilthoven, The Netherlands
- Gary Wnorowski, B.S., Laboratory Director, Product Safety Labs, East Brunswick, NJ

The following ICCVAM agency representatives were present:

- Dr. George Cushmac (Acute Toxicity Working Group; ATWG), U.S. Department of Transportation
- Dr. Kailash Gupta (ATWG), Consumer Product Safety Commission
- Dr. David Hattan, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration
- Dr. Richard Hill, (ICCVAM Co-Chair & ATWG), U.S. Environmental Protection Agency
- Ms. Vera Hudson, National Library of Medicine
- Dr. Devaraya Jagannath, Center for Veterinary Medicine (CVM), Food and Drug Administration
- Dr. William Stokes (ICCVAM Co-Chair & ATWG), National Institute of Environmental Health Sciences
- Dr. Kenneth Weber, National Institute for Occupational Safety and Health
- Dr. Errol Zeiger, National Institute of Environmental Health Sciences

The following members of the ICCVAM Acute Toxicity Working Group (ATWG) were present:

- Dr. Byron Backus, U.S. Environmental Protection Agency
- Mr. David Farrar, U.S. Environmental Protection Agency
- Dr. Roger Gardner, U.S. Environmental Protection Agency
- Dr. Masih Hashim, U.S. Environmental Protection Agency
- Dr. Elizabeth Margosches, U.S. Environmental Protection Agency
- Dr. Jeanie McAndrew, U.S. Environmental Protection Agency
- Dr. Debbie McCall, U.S. Environmental Protection Agency
- Dr. John Redden, U.S. Environmental Protection Agency
- Dr. Amy Rispin, U.S. Environmental Protection Agency
- Dr. Roy Sjoblad, U.S. Environmental Protection Agency
- Dr. Patrick Swann, Food and Drug Administration

The following members of the NICEATM Staff were present:

- Ms. Loretta Frye, National Institute of Environmental Health Sciences
- Mr. Brad Blackard, ILS, Inc.
- Ms. Sue Brenzel, ILS, Inc.
- Dr. Finis Cavender, ILS, Inc.
- Dr. Tom Goldsworthy, ILS, Inc.
- Ms. Christina Inhof, ILS, Inc.
- Ms. Linda Litchfield, ILS, Inc.
- Dr. Barry Margolin ILS, Inc.
- Dr. Ray Tice, ILS, Inc.

The following members of the public were present:

- Diane Beal, U.S. Environmental Protection Agency
- Dr. Gregg Carr, Procter and Gamble
- Eric Wilson, People for the Ethical Treatment of Animals (PETA)
- Jacqueline Russell, U.S. Environmental Protection Agency
- Nicholas Mastrotta, U.S. Environmental Protection Agency
- Carolyn Lingemen, Bethesda Environmental Health
- Monica Vegarra, Covance
- Martin Stephen, Humane Society of the U.S.
- Dr. Katherine Stitzel, Procter and Gamble
- Merrill Tisdell, Novartis
- Ann Marie Gebhart, UL
- Jean Holmes, U.S. Environmental Protection Agency
- Debbie Vich, DuPont
- Carol Finlay, DuPont
- Penny Fenner-Crisp, U.S. Environmental Protection Agency
- Roy Sjoblad, U.S. Environmental Protection Agency
- W.T. Meyer, U.S. Environmental Protection Agency
- Susan Makris, U.S. Environmental Protection Agency
- Jeff Ferguson, Rohm & Haas
- Sara Thurin Rollin, Bureau of Natural Affairs, Inc. (BNA)
- Lee Hofmann, U.S. Environmental Protection Agency
- Mario Styliano, U.S. Environmental Protection Agency
- Andrew Rowan, Humane Society of U.S.
- Liesel Wolff, PETA

Introductions

Dr. Curtis Klaassen, co-chair, called the meeting of the Special Emphasis Panel (SEP) for the independent peer review of the revised UDP to order at 8:36 a.m. and asked each attendee to state their name and affiliation. Dr. Klaassen informed the participants that the public would be given the opportunity to speak, that each speaker from the public would be limited to seven (7)

minutes, and that anyone addressing the group to please state their name for the benefit of the transcriptionist.

Dr. William Stokes read the Statement of Conflict of Interest and explained policies and procedures regarding confidentiality and avoidance of conflict of interest situations.

Welcome from the Acting Director of the Environmental Toxicology Program, NIEHS

Dr. Chris Portier thanked the ICCVAM participating agencies and the peer review panel (Panel) for their efforts. He presented an overview of the National Toxicology Program (NTP) and delineated several NTP initiatives associated with alternatives to traditional toxicity testing, including toxicogenomics, transgenic models, structure activity relationships, and mechanism-based mathematical modeling and computer simulation.

Introduction to ICCVAM and the ICCVAM Test Method Review Process

Dr. William Stokes, ICCVAM Co-Chair and Director of NICEATM, presented the background and history of ICCVAM and NICEATM and the process and procedures for evaluation of the Up-and-Down Procedure. He discussed the role of the ICCVAM Committee, its expert subgroup (Acute Toxicity Working Group [ATWG]), the Panel, and Public Law 103-43. This law directed the NIEHS to develop and validate alternative methods that can reduce or eliminate the use of animals in acute or chronic toxicity testing, establish criteria for the validation and regulatory acceptance of alternative testing methods, and recommend a process through which scientifically validated alternative methods can be accepted for regulatory use.

Criteria and processes for validation and regulatory acceptance were developed in conjunction with 13 other Federal agencies and programs with broad input from the public. These are described in the document "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods," NIH Publication 97-3981, NIEHS, 1997. This document is available on the internet at:

<http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf>

NIEHS and 13 other Federal regulatory and research agencies and programs subsequently established ICCVAM in a collaborative effort. The Committee's functions include the coordination of interagency reviews of toxicological test methods and communication with stakeholders throughout the process of test method development and validation, keeping in mind the 3 Rs (refinement, reduction, and replacement) of animal use.

The following Federal regulatory and research agencies and organizations are participating in this effort:

- Consumer Product Safety Commission
- Department of Defense
- Department of Energy

- Department of Health and Human Services
 - Agency for Toxic Substances and Disease Registry
 - Food and Drug Administration
 - National Institutes of Health
 - ❖ National Cancer Institute
 - ❖ National Institute of Environmental Health Sciences
 - ❖ National Library of Medicine
- National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention
- Department of the Interior
- Department of Labor
 - Occupational Safety and Health Administration
- Department of Transportation
 - Research and Special Programs Administration
- Environmental Protection Agency

Independent peer review is an essential prerequisite for consideration of a method for regulatory acceptance (NIEHS, 1997). The Panel was charged with evaluating and developing a consensus on the usefulness and limitations for each of the tests described in the UDP (Primary Test, Limit Test, and Supplemental Test) as a replacement for the OECD TG 401. The proposed test method and results of the peer review will be forwarded by ICCVAM to federal agencies for consideration. Federal agencies will determine the regulatory acceptability of the method according to their mandates.

Summary of Current Agency Requirements for Acute Oral Toxicity Data

Dr. Amy Rispin spoke on behalf of regulatory agencies regarding the needs for acute toxicity information for hazard classification and labeling and risk mitigation in the U.S. She presented an overview of the history and current agency regulations with regard to acute toxicity testing guidelines. Dr. Rispin stated that in 1999, OECD agreed that TGs 420, 423, and 425 should be updated and refined to meet the regulatory needs of member countries. These methods should include determination of slope, confidence intervals, and data to support classification and/or assessment of acute toxicity at a minimum of 5 mg/kg and a maximum of 5,000 mg/kg. Additionally, OECD member countries have been involved in international negotiations to characterize a harmonized scheme of classification for all health effect endpoints, to encourage the use of single sexes in testing, to take advantage of sequential dosing, to utilize appropriate statistical methods in these alternative guidelines, and to incorporate and use data from well-designed sighting studies.

The revisions to the UDP were intended to improve the performance of the basic UDP for a variety of chemicals and implement the recommendations made at a March 1999 OECD meeting in which discussions were aimed at alternative methods to TG 401. With increased dosing intervals, the Primary Test in the revised UDP method functions both as a range-finding test and a main test. With the revision, the limit dose of the test was extended to 5,000 mg/kg and sequential dosing was incorporated into all three tests (Primary, Limit, and Supplemental). Dr. Rispin added that the starting dose levels were evaluated to ensure that the test performed well

with new globally harmonized classification limits. Complementary testing can be conducted for slope and confidence intervals, by using the results of the Primary Test and the Supplemental Test. Additionally, the latest humane practices for animal handling and testing were incorporated.

Overview of the Revised Up-and-Down Procedure

Dr. Katherine Stitzel described the three test procedures (Primary, Limit, and Supplemental) outlined in the UDP guideline and provided background on the revised UDP procedure. She explained that the UDP is more useful when a point estimate of LD50 or an estimate of slope is needed, and discussed the requirements for achieving a point estimate of the LD50. The Primary Test provides an estimate of the LD50, the Limit Test indicates whether the LD50 is above or below the limit dose, and the Supplemental Test estimates the slope and the confidence interval. Information on the three test procedures may be found in the UDP background review document (BRD) and other supporting materials on the internet at <http://iccvam.niehs.nih.gov/methods/udpdocs/AllBRDlk.pdf>

Panel Presentations on Protocol and Tests of the UDP

Dr. Curtis Klaassen stated that the meeting would proceed with presentation of reports from the four sections charged with evaluation of the UDP: General Protocol Considerations, the Primary Test, Limit Test, and Supplemental Test Sections.

General Protocol Considerations

Dr. Janice Kuhn, the section coordinator, reviewed the general protocol for the three tests (Primary, Limit, and Supplemental). Section members included Ms. Kimberly Bonnette and Mr. Gary Wnorowski.

Dr. Kuhn explained that the role of this section was to offer a practical, laboratory-based perspective to the UDP. The assigned tasks were to evaluate the protocol, the level of ambiguity in the guideline, the practicality of the guideline in a laboratory setting, and the possibility of obtaining acute toxicity information without incurring undue increases in time or expense.

The Section concluded that the proposed test method protocol was generally adequate, but recommended the following additions and/or changes:

- The use of either sex (all males or all females) should be permitted unless information is available suggesting that one sex is more sensitive;
- The use of constant volume or constant concentration of the test material during administration should be allowed;
- All reference to littermates should be excluded from the Guideline;
- Animals of 8 to 12 weeks of age should be used;
- Individual animal body weights on the day of dosing must be within 20% of the mean body weight for all animals dosed throughout the study;

- Additional guidance that incorporates how to use all pre-start data (e.g., *in vitro* test results, physical and chemical properties) should be provided in the Guideline;
- The overall usefulness of information (e.g., clinical signs, time course of effects, target organs, pathology, etc.) gained beyond the LD50 should be emphasized in the Guideline; and
- The Guideline should be reorganized to improve clarity.

The conclusion of this section was that the revised UDP protocol, with minor adjustment, could replace TG 401, but that this replacement would bring an increase in costs and complexity. There was agreement with this conclusion and recommendations by the Panel members.

Revised Up-and-Down Procedure Primary Test

Dr. Wallace Hayes, the section coordinator, presented the analysis and conclusions reached by the Primary Test method reviewers, which included Drs. Bas Blaauboer, Robert Copeland, Nigel Stallard, and Mr. John Reeve.

With regard to the revised UDP Primary Test, the Section recommended that the Guideline would be improved with the following additions/revisions:

- The scientific basis should be presented in the Guideline;
- The Guideline should include a description of how historical data should be used to decide when to use the UDP Primary Test, the UDP Limit Test, or not to conduct any test;
- Additional guidance on the starting rule and a justification of the default starting dose of 175 mg/kg should be discussed in the Guideline;
- An improved description of stopping rule #3 should be included in the Guideline;
- User-friendly, validated software for test use or access to such software should be provided;
- In the Guideline, stopping rule #1 of the UDP Primary Test and the UDP Limit Test should be harmonized;
- In the Guideline, the term “half-log” units should be used throughout rather than the approximate dose progression factor of 3.2;
- Since no formal *in vivo* validation has been reported for the revised UDP Primary Test, at a minimum, a practicability evaluation of the revised test should be conducted (an appropriate working group should consider the design of this evaluation);
- In the Guideline, the overall usefulness of information (e.g., clinical signs, time course of effects, target organs, pathology, etc.) gained beyond the LD50 in the revised UDP Primary Test should be emphasized;
- The term “slope” should be defined in the Guideline; and
- The Guideline should state that any suitable statistical LD50 estimate method (e.g., isotonic regression) may be used.

The conclusion of this Section was that the revised UDP Primary Test would provide the same and possibly additional information when compared to TG 401, and that the Primary Test can replace TG 401 for classification purposes with the use of fewer animals. There was agreement with this conclusion and recommendations among the Panel members.

Public Comment Session

Mr. Mario Stylianou from the National Institutes of Health, the National Heart, Lung and Blood Institute described an additional method of estimating the LDp by using the maximum likelihood method modified as an isotonic regression estimate. When using the modified isotonic estimate, no estimate of *sigma* is needed. He stated that the use of the modified isotonic estimate also provided an estimate of the dose-response curve and that utilization of a statistical program reduces the level of complexity.

Dr. Andrew Rowan of the Humane Society of the U.S. stated that the assumption that the LD50 is a necessity was discouraging and that no precision exists with the LD50. Dr. Rowan challenged the Panel to determine the underlying assumptions that this test method is better than the previous and that the results are accurate.

Ms. Liesel Wolf of PETA (People for the Ethical Treatment of Animals) read a written commentary on behalf of Mary Beth Sweetland, the director of research investigations and the vice president of PETA. These written comments are included as an appendix. Ms. Wolf stated that the U.S. EPA remains one of the main obstacles to the OECD deletion of the *in vivo* LD50 test.

Dr. Martin Stephens, Humane Society of the U.S., stated that animal protectionists were concerned with the number of animals needed for the Revised UDP and that the quest for precision seemed more important than the protection of animals. He expressed concern over the males being bred and not used for testing and that the maximum dose level was increased from 2,000 to 5,000 mg/kg, thereby increasing distress levels in animals. Further concern was expressed with starting at high dose levels and then subsequently decreasing the dose levels. Dr. Stephens also called on the Federal agencies to provide information to interested parties on the extent of testing conducted.

Revised Up-and-Down Procedure Limit Test

Dr. George Alexeeff, section coordinator, presented the analysis and conclusions reached by the test method performance section reviewers, which included Drs. A.A.J. van Iersel and Robert Condon.

With regard to the revised UDP Limit Test, the Panel recommended that:

- The scientific basis and rationale should be added to the Guideline;
- Additional discussion of the applicability of the revised UDP Limit Test in the strategy of hazard or safety assessment should be included in the Guideline (a flow chart with decision criteria covering the complete testing scheme might be an efficient way to attain this goal);
- Consideration should be given to reorganizing the Guideline to improve clarity;
- Clarification of the selection of the limit dose would be helpful in the Guideline and the BRD;
- Additional calculations to justify the benefits of the revised UDP Limit Test would be helpful (i.e., the document should provide probability estimates for accuracy using criteria that

compare the revised UDP Limit Test to OECD TG 401 to clearly delineate the benefits, and the document should provide probability estimates for accuracy using more stringent criteria to determine if a further reduction in the number of animals tested is possible);

- The value of the revised UDP Limit Test would be improved if additional calculations were conducted regarding the probability for correct classification using other decision criteria; and
- The different stopping rules for the upper limit dose in the revised UDP Primary and Limit Tests may cause confusion and additional explanation in the BRD is suggested to address this issue.

The conclusion of this Section was that the Limit Test may be performed when it is necessary to determine if the LD50 is above a defined limit (2,000 or 5,000 mg/kg). There was agreement with this conclusion and recommendations among the Panel members.

Supplemental Test

Dr. Bob Scala, the section coordinator, presented the analysis and conclusions reached by the supplemental test section reviewers, which included Drs. Nancy Flournoy, Phil Botham, Wyman Dorough, and Charles Hastings.

With regard to the UDP Supplemental Test, this Section recommended that:

- Regulatory data needs currently addressed by estimation of the slope and confidence interval derived from acute oral toxicity studies in the rat and other species need to be more clearly defined; and
- Consideration should be given as to whether the slope and confidence interval are the most appropriate parameters for risk assessment or whether risk assessment needs can be addressed more directly. For example, if estimates of points on the dose-response curve well below the median lethal dose are needed in environmental risk assessment, more efficient methods should be considered.

The UDP Supplemental Test for slope and confidence interval was not recommended for adoption. The Panel concluded that they were unable to evaluate the utility of the test because sufficient information regarding the use of the resulting data was not provided.

Peer Review Panel Conclusions

Co-chairperson, Dr. Diane Gerken, led the discussion and voting regarding the two major questions posed to the Panel.

The Panel was charged with separately addressing the following two questions for each of the three tests:

1. Has the revised UDP been evaluated sufficiently and is its performance satisfactory to support its adoption as a substitute for the currently accepted UDP

(OECD, 1998), and as a substitute for the traditional LD50 test for acute oral toxicity (U.S. EPA Health Effects Guidelines, OPTTS 870.1100; OECD, 1987)?

2. With respect to animal welfare, does the revised UDP adequately consider and incorporate where scientifically feasible, procedures that refine, reduce, and/or replace animal use?

In response to these questions, the Panel concluded that:

- The performance of the revised UDP Primary Test is satisfactory and exceeds the performance of OECD TG 401 in providing, with fewer animals, both an improved estimate of the LD50 for the purpose of hazard classification and more accurate information on acute toxicity. In particular, the use of 0.5 log units for dose spacing is reasonable and appropriate based on experience and the results of computer simulations. Three disadvantages of the revised UDP Primary Test recognized by the Panel were: a) the increased length of time needed to conduct a study; b) the increased costs per test material evaluated; and c) the increased complexity of the protocol.
- The revised UDP Limit Test at 2000 or 5000 mg/kg is expected to perform as well as or better than the Limit Test in OECD TG 401, with a reduction in the number of animals needed to conduct a test.
- The UDP Supplemental Test for slope and confidence interval is not recommended for adoption. The Panel was unable to evaluate the utility of the test because sufficient information regarding the use of the resulting data was not provided. As a consequence, any impact on animal use was not assessed.

The revised UDP Primary Test and the revised UDP Limit Test will reduce the number of animals used, but will not replace the use of animals. The Panel could not reach a consensus on the overall issue of refinement. However, the OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (OECD, 1999), referenced in the revised UDP Guideline, provides an element of refinement.

Dr. Stokes on behalf of ICCVAM and its participating agencies thanked the Panel for their thoughtful deliberations and careful evaluation of the test method and background materials.

Dr. Klaassen adjourned the meeting at 5:10 p.m.